

AUTHOR'S VIEW

RAS-MAPK signaling influences the efficacy of ALK-targeting agents in lung cancer

Gorjan Hrustanovic^{a,b} and Trever G. Bivona^{a,b}

^aDivision of Hematology and Medical Oncology, University of California San Francisco, San Francisco, CA, USA; ^bHelen Diller Family Comprehensive Cancer Center, University of California San Francisco, San Francisco, CA, USA

ABSTRACT

ALK (anaplastic lymphoma kinase) inhibitors are approved in for ALK gene rearrangement positive (ALK+) lung cancer, but resistance remains a challenge. We discovered that RAS-RAF-MEK-ERK signaling controls the ALK inhibitor response in ALK+ lung cancer and is critical for ALK inhibitor resistance. Upfront ALK-MEK inhibitor polytherapy may enhance response and forestall resistance.

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The identification of oncogenic alterations that promote cancer growth and the development of oncogene-targeted therapies have revolutionized the treatment of for many cancer patients.^{1,2} Despite the success of oncogene-targeted therapy, resistance remains a challenge.² Both primary and acquired drug resistance frequently occur in patients and are often lethal. The molecular determinants of resistance remain unknown in many cases. This is a critical knowledge gap, as understanding of the molecular events that promote innate and acquired resistance would provides biological insight into cell signaling and offers therapeutic strategies to thwart resistance and enhance clinical outcomes.

Our recent work focused on resistance to targeted therapies acting against the oncogenic ALK gene rearrangement (*EML4-ALK* (; *EML4*, echinoderm microtubule associated protein like 4) in lung cancers (referred to as ALK+ cancer).³ ALK inhibitors (crizotinib and ceritinib) are initially, yet but only transiently, effective in some, but not all, ALK+ mutant patients because of resistance.^{4,5} One promising approach to overcome such resistance is the use of a rational upfront combination therapy targeting an oncogenic protein plus a critical downstream pathway. This approach has been successful in *BRAF*^{V600E}-mutant melanoma patients, in whom upfront targeting of oncogenic BRAF plus its downstream effector MEK yields superior clinical responses when compared to BRAF or MEK inhibitor monotherapy.⁶ However, which effector pathway is most critical to as a target in cancers with an oncogenic receptor kinase, such as *EML4-ALK*, is unclear since receptor kinases typically activate multiple pathways.³ We hypothesized that dissection of the pathway dependencies in ALK+ lung cancer cells may reveal the individual pathway that is essential for ALK+ lung cancer cell survival and is the critical determinant of ALK inhibitor response.

To explore this hypothesis, we used a pharmacologic and genetic strategy in which we suppressed or activated the 3 major *EML4-ALK* effector pathways:, RAF-MEK-ERK

(MAPK, mitogen activated protein kinase (MAPK), PI3K (phosphoinositide 3-kinase (PI3K)/AKT, and JAK/STAT (Janus kinase/signal transducer and activators of transcription) signaling.³ We found that RAF-MEK-ERK signaling was uniquely required for ALK+ lung cancer cell survival (Fig. 1); Whereas whereas inhibition of MEK or ERK was lethal in ALK+ cells, inhibition of PI3K/AKT or JAK/STAT had no effect. We discovered that *EML4-ALK* engaged all 3 RAS isoforms (H-, N-, K-RAS) to drive RAF-MEK-ERK signaling. This molecular link between *EML4-ALK* and RAS required the HELP domain in the *EML4* component of *EML4-ALK*, which . This HELP domain in *EML4* was essential for proper intracellular localization of *EML4-ALK* and downstream RAS-RAF-MEK-ERK signaling. We found that ALK+ lung cancer cells and patient tumors that develop resistance to ALK inhibitor treatment reactivate MEK-ERK signaling by 2 mechanisms:. Firstfirst, some tumors harbor copy number gain of wild- type (WT) KRAS and. Secondsecond, patient tumors downregulated the MEK-ERK phosphatase DUSP6, resulting in persistent pathway activation. Both events cause activation of MEK-ERK signaling during ALK inhibitor treatment, thus driving resistance. Inhibition of both MEK and ALK enhanced the initial therapy response and prevented resistance in ALK+ cells, *in vitro* and *in vivo*. These data uncover MEK-ERK pathway activation as a hallmark of resistance to ALK targeted therapy and suggest that upfront ALK and MEK co-inhibition may enhance response and patient survival.

Open questions

Our findings prompt new avenues for research. First, the findings raise the possibility that MEK inhibition may substantially minimize or eliminate resistance in patients with ALK+ lung cancer patients. Efforts are focused on translating the findings into a clinical trial testing ALK inhibitor treatment with a sub-maximal dose of MEK inhibitor in ALK+ patients. The

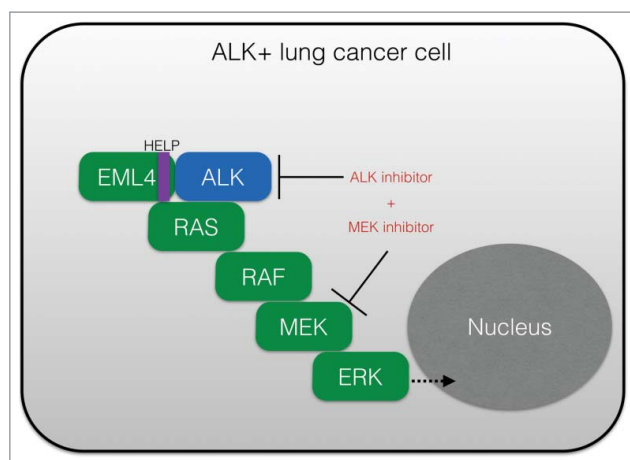


Figure 1. RAS-MAPK signaling promotes cancer cell survival and resistance to ALK-targeting agents. Shown is a schematic of the essential role of RAS-RAF-MEK-ERK (RAS-MAPK) signaling in ALK+ (*EML4-ALK* positive) tumor cells, and the rational co-targeting strategy to block both ALK and MEK to enhance anti-tumor response. MAPK, mitogen activated protein kinase; ALK, anaplastic lymphoma kinase; HELP, HELP domain in EML4 (echinoderm microtubule associated protein like 4 (EML4)).

use of a sub-maximal dose of the MEK inhibitor is attractive given the clinical toxicity observed with full-dose MEK inhibition in other patients. Our preclinical data indicate that sub-maximal MEK inhibition is sufficient to enhance the response and eliminate ALK inhibitor resistance, all with improved safety in mice compared to each full-dose monotherapy. Combined ALK plus MEK inhibition may be a viable approach in lung cancer patients, similar to melanoma.⁶ Yet, the success of this treatment strategy will require careful phase I and pharmacodynamic clinical studies to determine the optimal combination regimen that is safe and effective.

Second, on a fundamental level the findings provide a rationale to investigate the molecular basis of the connection between ALK and RAS-RAF-MEK-ERK signaling. We found that EML4-ALK is expressed on an intracellular locale that is yet to be fully determined, and that this localization is critical to for engagement of RAS and downstream RAF-MEK-ERK signaling. The Identification of the factors underlying this compartment-specific ALK-RAS regulation and coupling areis critical to understand and a focus of ongoing efforts.

Third, the study reveals the utility value of deciphering pathway dependencies in cancer cells with a particular oncogenic receptor kinase. The development of rational upfront combination therapies will require prioritization, as empiric testing of all possible combination regimens is not possible in patients and could lead to unnecessary toxicity and lack of efficacy. Our

approach to prioritize polytherapies may be applied more broadly in cancers with oncogenic receptor kinases such as mutant *EGFR* (epidermal growth factor receptor) or *ROS1* gene rearrangements.⁷ to define the rational polytherapy mostly likely to maximize clinical responses.

Implications

Our study indicates that RAS-MAPK dependence is a hallmark of ALK+ lung cancers. The data provide a rational basis for upfront testing of a MEK inhibitor (at sub-maximal dose) plus an ALK inhibitor to enhance the magnitude and duration of the anti-tumor response in patients. The findings provide a rationale to explore the cell biologicalular basis of oncogenic receptor kinase signaling, with implications for the design of rational upfront targeted polytherapies in for cancer.

Disclosure of potential conflicts of interest

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